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EP04/11054

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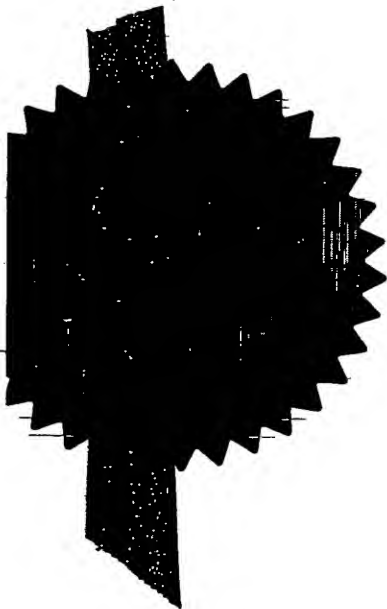
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# Request for grant of a patent

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3 OCT 2003

The Patent Office

Cardiff Road  
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1.	Your reference	4-33373P1		
2.	Patent application number (The Patent Office will fill in this part)	0323204.8		
3.	Full name, address and postcode of the or of each applicant (underline all surnames)	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND		
	Patent ADP number (if you know it)			
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND	07125487005	
4.	Title of invention	Organic Compounds		
5.	Name of your agent (If you have one)	Craig McLean		
	"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	Novartis Pharmaceuticals UK Limited Patents and Trademarks Wimblehurst Road Horsham, West Sussex RH12 5AB		
	Patents ADP number (if you know it)	07181522002 ✓		
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day/month/year)	
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:	Yes		
	a) any applicant named in part 3 is not an inventor, or			
	b) there is an inventor who is not named as an applicant, or			
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## Patents Form 1/77

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Continuation sheets of this form

Description 16 /

Claim(s) 3 /

Abstract

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10. If you are also filing any of the following, state how many against each item.

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Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*) 1 /

Request for substantive examination (*Patents Form 10/77*)

Any other documents  
(please specify)

11.

I/We request the grant of a patent on the basis of this application

Signature

Date



Craig McLean

3<sup>rd</sup> October 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr. Trevor Drew

01403 323069

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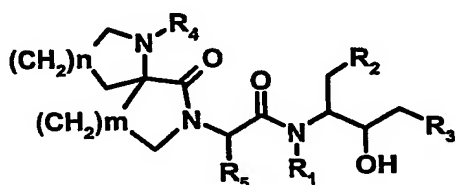
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DUPLICATE

Organic Compounds

The present invention relates to novel 2-(6-oxo-1,7-diaza-spiro[4.4]non-7-yl)-propionamides, their preparation, their use as pharmaceuticals and pharmaceutical compositions containing them.

More particularly the invention provides compounds of formula I



wherein

$R_1$  is hydrogen or  $(C_{1-4})$ alkyl,

$R_2$  is optionally substituted  $(C_{1-8})$ alkyl,  $(C_{3-7})$ cycloalkyl,  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl, aryl or heteroaryl,

$R_3$  is  $CH(R_6)CONR_aR_b$  or  $(CH_2)_kNR_cR_d$ , wherein

$k$  is 0, 1 or 2,

$R_a$ ,  $R_b$ ,  $R_c$  and  $R_d$ , independently, are hydrogen or optionally substituted  $(C_{1-8})$ alkyl,  $(C_{3-7})$ cycloalkyl,  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl, aryl, aryl $(C_{1-4})$ alkyl, heteroaryl, heteroaryl $(C_{1-4})$ alkyl, 4-chromanyl, 1,2,3,4-tetrahydro-quinolin-4-yl, 1,2,3,4-tetrahydro-naphthalen-1-yl, thiochroman-4-yl-1,1-dioxide, 4-isochromanyl, 1,2,3,4-tetrahydro-isoquinolin-4-yl, thioisochroman-4-yl-1,1-dioxide, 1,1-dioxo-1,2,3,4-tetrahydro-1 $\lambda^6$ -benzo[e][1,2]thiazin-4-yl, 1,1-dioxo-3,4-dihydro-1H-1 $\lambda^6$ -benzo[c][1,2]oxathiin-4-yl, 2,2-dioxo-1,2,3,4-tetrahydro-2 $\lambda^6$ -benzo[c][1,2]thiazin-4-yl or 2,2-dioxo-3,4-dihydro-2H-2 $\lambda^6$ -benzo[e][1,2]oxathiin-4-yl, or

$R_a$ , and  $R_b$ , or  $R_c$  and  $R_d$ , together with the nitrogen to which they are attached, form an optionally substituted pyrrolidiny, piperidino, morpholino or piperazinyl group, and

$R_6$  is  $(C_{1-8})$ alkyl,  $(C_{1-4})$ alkoxy $(C_{1-4})$ alkyl,  $(C_{3-7})$ cycloalkyl or  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl,

$R_4$  is hydrogen or optionally substituted  $(C_{1-8})$ alkyl,  $(C_{1-4})$ alkoxy $(C_{1-4})$ alkyl,  $(C_{3-7})$ cycloalkyl, or  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl,  $(C_{1-8})$ alkenyl or  $(C_{3-7})$ cycloalkoxy $(C_{1-4})$ alkyl,

$R_5$  is hydrogen or optionally substituted  $(C_{1-4})$ alkyl, and

m and n, independently, are 1 or 2,

in free base or acid addition salt form.

On account of the asymmetrical carbon atoms present in the compounds of formula I and their salts, the compounds may exist in optically active form or in form of mixtures of optical isomers, e.g. in form of racemic mixtures. All optical isomers and their mixtures including the racemic mixtures are part of the present invention.

**Substituents on above defined non-aromatic groups** are selected from hydroxy, halogen, carbamoyl, carboxy, hydroxy(C<sub>1-4</sub>)alkyl, (C<sub>1-4</sub>)alkoxy, (C<sub>1-4</sub>)alkoxy(C<sub>1-4</sub>)alkyl, (C<sub>1-4</sub>)alkoxy(C<sub>1-4</sub>)alkoxy, (C<sub>1-4</sub>)alkylsulfanyl, (C<sub>1-4</sub>)alkoxycarbonyl, (C<sub>1-4</sub>)alkylcarbonyloxy, (C<sub>1-4</sub>)alkylcarbonyl, (C<sub>1-4</sub>)alkylsulfonyl, cyano, oxo, hetero(C<sub>3-7</sub>)cycloalkyl, optionally substituted aryl or heteroaryl. (C<sub>3-7</sub>)cycloalkyl or hetero(C<sub>3-7</sub>)cycloalkyl groups can also be fused with an additional (C<sub>3-7</sub>)cycloalkyl, hetero(C<sub>3-7</sub>)cycloalkyl, or an aromatic or heteroaromatic ring.

**Substituents on above defined aromatic or heteroaromatic groups** are selected from halogen, hydroxy, cyano, nitro, trifluoromethyl, benzyloxy, phenoxy, SO<sub>2</sub>NH<sub>2</sub>, NHSO<sub>2</sub>(C<sub>1-3</sub>)alkyl, carboxy, (C<sub>1-4</sub>)alkyloxycarbonyl, carbamoyl, (C<sub>1-4</sub>)alkylcarbamoyl, (C<sub>1-4</sub>)alkylsulfonyl, (C<sub>1-4</sub>)alkylcarbonyloxy, (C<sub>1-4</sub>)alkylcarbonyl, (C<sub>1-4</sub>)alkyl, (C<sub>1-4</sub>)alkoxy, hydroxy(C<sub>1-4</sub>)alkyl, aryl, heteroaryl or an optionally substituted amino group.

**Substitutents on amino or carbamoyl groups** can be one or two groups selected from (C<sub>1-4</sub>)alkyl, (C<sub>1-4</sub>)alkoxy(C<sub>1-4</sub>)alkyl, (C<sub>1-4</sub>)alkoxycarbonyl, aryl(C<sub>1-4</sub>)alkyloxycarbonyl or heteroaryl(C<sub>1-4</sub>)alkyloxycarbonyl.

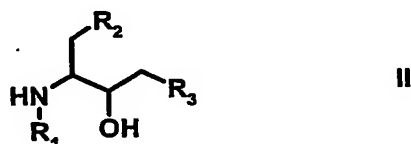
**Aryl** is an aromatic 6-membered ring optionally mono-, di- or tri-substituted by, independently, hydroxy, cyano, trifluoromethyl, carboxy, (C<sub>1-4</sub>)alkyloxycarbonyl, (C<sub>1-4</sub>)alkylcarbamoyl, (C<sub>1-4</sub>)alkylsulfonyl, (C<sub>1-4</sub>)alkylcarbonyloxy, (C<sub>1-4</sub>)alkylcarbonylamino, (C<sub>1-4</sub>)alkylcarbonyl, (C<sub>1-4</sub>)alkyl, (C<sub>1-4</sub>)alkoxy or hydroxy(C<sub>1-4</sub>)alkyl. Aryl groups can also be fused with a (C<sub>3-7</sub>)cycloalkyl, hetero(C<sub>3-7</sub>)cycloalkyl or additional aromatic or heteroaromatic ring (e.g. to form a naphthyl, quinoliny or indolyl group).

**Heteroaryl** is an aromatic 5- or 6- membered ring in which 1, 2 or 3 atoms are heteroatoms independently selected from O, N and S. Heteroaryl is for example 1-methyl-1H-pyrrol-2-yl or 1H-imidazol-2-yl. It can also be fused with a cycloalkyl or additional aromatic or heteroaromatic ring (e.g. to form a quinolinyl, or indolyl group).

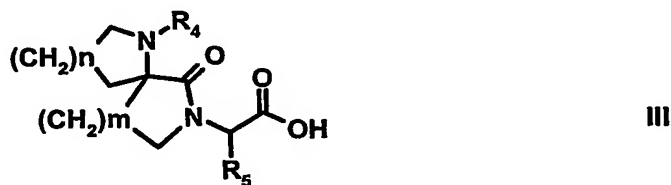
**Halogen** denotes fluorine, bromine, chlorine or iodine.

Any alkyl, alkenyl or alkoxy group is straight or branched.

In a further aspect, the invention provides a process for the production of the compounds of formula I and their salts, comprising the steps of acylating a compound of formula II



wherein  $R_1$ ,  $R_2$  and  $R_3$  are as defined above, with an acid of formula III



wherein  $R_4$ ,  $R_5$ ,  $m$  and  $n$  are as defined above, or an activated form thereof, and recovering the so obtained compound of formula I in free base or acid addition salt form.

The reaction can be effected according to conventional methods, for example as described in the examples.

The compounds of formula I can also be produced by further conventional processes, e.g. as described in the examples.

The starting materials of formulae II and III are known or may be prepared according to conventional procedures starting from known compounds, for example as described in the examples.

Working-up the reaction mixtures and purification of the compounds thus obtained may be carried out in accordance to known procedures.

Acid addition salts may be produced from the free bases in known manner, and vice-versa.

Compounds of formula I and their pharmaceutically acceptable acid addition salts, hereinafter referred to as agents of the invention, exhibit valuable pharmacological properties when tested in vitro and in animals, and are therefore useful as pharmaceuticals.

The agents of the invention are inhibitors of aspartic proteases and can be used for the treatment of disorders involving processing by such enzymes. Particularly they inhibit beta-secretase and as such inhibit the generation of beta-amyloid and the subsequent aggregation into oligomers and fibrils.

#### **Test 1 Inhibition of human BACE**

Recombinant BACE (extracellular domain, expressed in baculovirus and purified using standard methods) at 6 nM concentration is incubated with test compound at various concentrations for 1 hour at room temperature in 100 mM acetate buffer, pH 4.5, containing 0.1 % CHAPS. Synthetic peptide substrate Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(DNP) is added to a final concentration of 3  $\mu$ M and increase in fluorescence is recorded at excitation of 325 nm and emission at 400 nm in a microplate spectro-fluorimeter for 20 minutes in 1-minute intervals. IC<sub>50</sub> values are calculated from percentage of inhibition of BACE-activity as a function of test compound concentration.

#### **Test 2 Inhibition of human BACE-2**

Recombinant BACE-2 (extracellular domain, expressed in baculovirus and purified using standard methods) at 2.5 nM concentrations incubated with test compound at various concentrations for 1 hour at room temperature in 100 mM acetate buffer, pH 4.5, containing 0.1 % CHAPS. Synthetic peptide substrate Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-

Lys(DNP) is added to a final concentration of 3  $\mu$ M and increase in fluorescence is recorded at excitation of 325 nm and emission at 400 nm in a microplate spectro-fluorimeter for 20 minutes in 1-minute intervals. IC<sub>50</sub> values are calculated from percentage of inhibition of BACE-2-activity as a function of test compound concentration.

### **Test 3 Inhibition of human Cathepsin D**

Recombinant cathepsin D (expressed as procathepsin D in baculovirus, purified using standard methods and activated by incubation in sodium formate buffer pH 3.7) is incubated with test compound at various concentrations for 1 hour at room temperature in 100 mM sodium formate buffer, pH 3.1. Synthetic peptide substrate Mca-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-D-Arg-NH<sub>2</sub> is added to a final concentration of 2  $\mu$ M and increase in fluorescence is recorded at excitation of 325 nm and emission at 400 nm in a microplate spectro-fluorimeter for 20 minutes in 1-minute intervals. IC<sub>50</sub> values are calculated from percentage of inhibition of cathepsin D-activity as a function of test compound concentration.

### **Test 4 Inhibition of cellular release of amyloid peptide 1-40**

Chinese hamster ovary cells are transfected with the gene for amyloid precursor protein. Cells are plated at a density of 8000 cells/well in a 96- well microtiter plate and cultivated for 24 hours in DMEM cell culture medium containing 10 % FCS. Test compound is added to the cells at various concentrations, and cells are cultivated for 24 hours in presence of test compound. Supernatants are collected, and concentration of amyloid peptide 1-40 is determined using sandwich ELISA. Potency of the compound is calculated from the percentage of inhibition of amyloid peptide release as a function of test compound concentration.

In at least one of the above-indicated tests, the agents of the invention show activity at concentrations below 20  $\mu$ M.

The agents of the invention are therefore useful e.g. for the treatment and/or prevention of neurological and vascular disorders related to beta-amyloid generation and/or aggregation such as neurodegenerative diseases like Alzheimer's disease, Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain-trauma, vascular amyloidosis, or cerebral haemorrhage with amyloidosis.

Some of the agents of the invention also inhibit BACE2 (beta-site APP-cleaving enzyme 2) or Cathepsin D, close homologues of the pepsin-type aspartyl proteases. Due to the correlation of BACE2 and CathD expression with a more tumorigenic and metastatic potential of tumor cells, such inhibitors are useful for the suppression of the metastasis process associated with tumor cells.

For the above-mentioned indications, the appropriate dosage will of course vary depending upon, for example, the compound employed, the host, the mode of administration and the nature and severity of the condition being treated. However, in general, satisfactory results in animals are indicated to be obtained at a daily dosage of from about 0.1 to about 100, preferably from about 1 to about 50 mg/kg animal body weight. In larger mammals, for example humans, an indicated daily dosage is in the range from about 10 to about 2000, preferably from about 10 to about 200 mg of an agent of the invention conveniently administered, for example, in divided doses up to four times a day or in sustained release form.

The agent of the invention may be administered by any conventional route, in particular enterally, preferably orally, for example in the form of tablets or capsules, or parenterally, for example in the form of injectable solutions or suspensions.

In accordance with the foregoing, the present invention also provides an agent of the invention, for use as a pharmaceutical, e.g. for the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation.

The present invention furthermore provides a pharmaceutical composition comprising an agent of the invention in association with at least one pharmaceutical carrier or diluent. Such compositions may be manufactured in conventional manner. Unit dosage forms contain, for example, from about 1 to about 1000, preferably from about 1 to about 500 mg of an agent of the invention.

The agents of the invention can be administered alone or in combination with other pharmaceutical agents effective in the treatment of conditions mentioned above.

The pharmaceutical combination may be in form of a unit dosage form, whereby each unit dosage will comprise a predetermined amount of the two components, in admixture with suitable pharmaceutical carriers or diluents. Alternatively, the combination may be in form of a package containing the two components separately, e.g. a pack or dispenser-device adapted for the concomitant or separate administration of the two active agents, wherein these agents are separately arranged.

Moreover the present invention provides the use of an agent of the invention, for the manufacture of a medicament for the treatment of any neurological and vascular disorders related to beta-amyloid generation and/or aggregation.

In still a further aspect the present invention provides a method for the treatment of any neurological and vascular disorders related to beta-amyloid generation and/or aggregation, in a subject in need of such treatment, which comprises administering to such subject a therapeutically effective amount of an agent of the invention.

The following examples illustrate the invention.

**Abbreviations:**

BOC	tert-butoxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate
DCM	dichloromethane
DMPU	N, N'-dimethylpropyleneurea
EDC.HCl	1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride
EtOAc	ethylacetate
h	hours
HCl	hydrochloric acid
HOBt	hydroxybenzotriazole
HPLC	high pressure liquid chromatography
LAH	lithium aluminum hydride
min	minutes
Mp	melting point
MS	mass spectroscopy
Rf	retention factor (TLC)

rt	room temperature
TBME	<i>tert</i> -butyl methyl ether
TBTU	O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-tetrafluoroborate
TFA	trifluoroacetic acid
THF	tetrahydrofuran

**Example 1: 4-(S)-Hydroxy-5-(S)-[2-(S)-(1-isobutyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

113 mg (0.4 mmol) 2-(S)-(1-isobutyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionic acid methyl ester are dissolved in 18 mL THF and treated with 2 mL of a 0.5 N aqueous LiOH solution and stirred at room temperature for four hours. The reaction mixture is cooled to 5°C and treated with an aqueous 1N HCl solution until a pH of 4 is reached. The solution is submitted to two consecutive azeotropic evaporations with 80 mL toluene and the residue dried under high vacuum, then taken up in 20 mL dichloromethane and stirred at room temperature for twenty hours with 117 mg (0.4 mmol) 5-(S)-amino-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide, 85 mg (0.44 mmol) EDC.HCl, 54 mg (0.4 mmol) HOBt and 0.17 mL triethylamine (1.2 mmol). The reaction mixture is quenched with 10 mL ice-cold saturated aqueous sodium bicarbonate solution, then extracted twice with dichloromethane. The combined organic phases are evaporated and the residue column chromatographed (silica gel, TBME/EtOAc/EtOH 49:59:2) to yield after evaporation of the pure fractions the desired product as a colorless resin.

MS(EI+)453 (M+1)

The starting materials can be prepared as described hereafter:

**a) 2-(S)-(1-isobutyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionic acid methyl ester**

260 mg (0.8 mmol) 7-(1-Methoxycarbonyl-(S)-ethyl)-6-oxo-1,7-diaza-spiro-(S)-[4.4]nonane-1-carboxylic acid *tert*-butyl ester is stirred in 3 mL of a 4N solution HCl in dioxane for three hours at room temperature, then evaporated. The residue is taken up in toluene and evaporated to dryness (twice), then taken up in 6 mL methanol, treated with 0.145 mL (1.6 mmol) isobutyraldehyde, 300 mg 3Å powdered molecular sieve and 100 mg (1.6 mmol) sodium cyanoborohydride and stirred overnight at room temperature. The reaction mixture is

treated with 4 mL saturated aqueous ammonium chloride solution and after 10 minutes with 8 mL saturated aqueous sodium bicarbonate, then extracted with EtOAc. The combined organic phases are evaporated and the residue column chromatographed (silica gel, EtOAc) to yield after evaporation of the corresponding fractions the desired product as a colorless oil.  
MS (ES<sup>+</sup>): 283 (M+1)

**b) 7-(1-Methoxycarbonyl-(S)-ethyl)-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester and 7-(1-Methoxycarbonyl-(S)-ethyl)-6-oxo-1,7-diaza-spiro-(S)-[4.4]nonane-1-carboxylic acid tert-butyl ester**

4.3 g (12 mmol) 2-[2-(1-Methoxycarbonyl-ethylamino)-(S)-ethyl]-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester are dissolved in 60 mL xylene and heated to 150°C for two hours. The reaction mixture is evaporated and the residue column chromatographed (silica gel, EtOAc/petroleum ether 3:2) to yield 1.85 g (46%) 7-(1-methoxycarbonyl-(S)-ethyl)-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester and 1.8 g (45%) 7-(1-methoxycarbonyl-(S)-ethyl)-6-oxo-1,7-diaza-spiro-(S)-[4.4]nonane-1-carboxylic acid tert-butyl ester. The absolute stereochemistry is confirmed by X-ray of a sample recrystallized in diisopropylether.

**c) 2-[2-(1-Methoxycarbonyl-(S)-ethylamino)-ethyl]-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester**

271 mg (1 mmol) 2-(2-Oxo-ethyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester and 154 mg (1.1 mmol) L-alanine methyl ester hydrochloride are suspended in 10 mL toluene and treated with 0.073 mL (1 eq.) triethylamine. The reaction mixture is stirred 10 minutes at room temperature and slowly evaporated in a rotary evaporator. The residue is taken up in 15 mL acetonitrile and 95 mg (1.5 mmol) sodium cyanoborohydride in 2 mL methanol added dropwise. Upon completion of the reaction (TLC, EtOAc/petroleum ether 4:1), the reaction mixture is evaporated and the residue taken up in ethyl acetate and treated with an ice-cold, saturated aqueous ammonium chloride solution, then extracted with ethyl acetate and saturated aqueous sodium bicarbonate. The combined organic phases are dried over sodium sulfate, filtered and evaporated to yield the crude desired product as a thick oil.

MS(EI<sup>+</sup>): 359 (M+1)

R<sub>f</sub>(EtOAc): 0.22

**d) 5-(S)-amino-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

32 mg (0.1 mmol) [1-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-(S)-phenyl-ethyl]-carbamic acid tert-butyl ester are stirred at room temperature for two hours in 1 mL of a 4N HCl solution in dioxane. The reaction mixture is evaporated to dryness, the residue taken up in toluene and evaporated to dryness twice before drying under high vacuum. The residue is taken up in 1 mL (excess) butylamine and stirred at 25°C overnight, then evaporated and the residue extracted twice with ethyl acetate and saturated sodium bicarbonate. The combined organic phases are evaporated, the crude desired product obtained quantitatively as a colorless resin and used without further purification.

MS(EI+): 293 (M+1).

The following compounds are obtained by a similar procedure:

**Example 1a: 5-(S)-[2-(S)-(1-Cyclopropylmethyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 541 (M+1)

**Example 1b: 5-(S)-[2-(S)-(1-Propyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 529 (M+1)

**Example 1c: 5-(S)-[2-(S)-(1-Phenyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 591 (M+1)

**Example 1d: 5-(S)-[2-(S)-(1-Phenyl-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 591 (M+1)

**Example 1e:** 4-(S)-Hydroxy-2-(R)-methyl-5-(S)-[2-(S)-(6-oxo-1-propyl-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-6-phenyl-hexanoic acid (2,2-dimethyl-propyl)-amide

MS(EI+): 543 (M+1)

**Example 1f:** 5-(S)-[2-(S)-(1-Cyclopropylmethyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid bicyclo[2.2.1]hept-exo-2-(R,S)-ylamide

MS(EI+): 579 (M+1)

**Example 2:** 5-(S)-[2-(S)-(1-Allyl-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide

52 mg (0.1 mmol) 4-(S)-Hydroxy-2-(R)-methyl-5-(S)-[2-(S)-(6-oxo-1,7-diaza-spiro-(R)-[4.4]non-7-yl)-propionylamino]-6-phenyl-hexanoic acid butyl amide hydrochloride, 28 mg potassium carbonate and 0.01 mL allyl bromide are stirred at room temperature for 65 hours in 3 mL DMF, then extracted with EtOAc and brine (twice). The combined organic phases are dried over sodium sulfate, evaporated and column chromatographed to yield the desired product as a light-colored resin.

MS(EI+): 527 (M+1)

The starting materials can be prepared as described hereafter:

**a) 4-(S)-Hydroxy-2-(R)-methyl-5-(S)-[2-(S)-(6-oxo-1,7-diaza-spiro-(R)-[4.4]non-7-yl)-propionylamino]-6-phenyl-hexanoic acid butyl amide hydrochloride**

170 mg (0.33 mmol) 7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester are dissolved in 1.5 mL (excess) butylamine and heated to 65°C under argon for two hours. The reaction mixture is evaporated, the residue taken up in toluene and evaporated to dryness, redissolved in 5 mL isopropanol, treated with 1 mL of a 6N HCl solution in isopropanol and stirred at room temperature for four hours, then evaporated, taken up in

toluene and evaporated again to yield the desired product, which is used without further purification.

MS (EI+) = 487 (M+1)

**b) 7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester and 7-{1-(S)-[1-(R)-(4-(S)-Methyl-5-oxo-tetrahydro-furan-2-(R)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester**

326 mg (1 mmol) 7-(1-(S)-Methoxycarbonyl-ethyl)-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester are dissolved in 15 mL THF, cooled to 10°C and treated with 7 mL (1.05 eq) of a 0.15 N THF solution of LiOH. After two hours stirring at room temperature, a 1 N aqueous HCl solution is added until a pH of 4 was reached, and the reaction mixture evaporated. The residue is taken up in toluene, evaporated to dryness and dried under high vacuum, then taken up in 20 mL dichloromethane and stirred for 18 hours after addition of 230 mg racemic 5-(1-amino-2-phenyl-ethyl)-3-methyl-dihydro-furan-2-one, 135 mg HOBt (1 mmol), 208 mg EDC.HCl (1.1 mmol) and 0.031 mL triethylamine (2.25 mmol). The reaction mixture is extracted with EtOAc and saturated aqueous sodium bicarbonate, the combined organic fractions are washed with brine, evaporated and column chromatographed (silica gel, EtOAc/diisopropylether 4:1) to yield 7-{1-(S)-[1-(S)-(4-(R)-methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester and 7-{1-(S)-[1-(R)-(4-(S)-methyl-5-oxo-tetrahydro-furan-2-(R)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester is white solids in respectively 33 and 30% yields.

The absolute stereochemistry is confirmed by comparison with optically pure material made from 5-(S)-(1-(S)-amino-2-phenyl-ethyl)-3-(R)-methyl-dihydro-furan-2-one.

MS(EI+): 514 (M+1)

The following compounds are obtained by a similar procedure:

**Example 2a: 5-(R)-[2-(S)-(1-Allyl-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-7-yl)-propionylamino]-4-(R)-hydroxy-2-(S)-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 527 (M+1)

**Example 2b:** 5-(R)-[2-(S)-(1-Allyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-4-(R)-hydroxy-2-(S)-methyl-6-phenyl-hexanoic acid butylamide

MS(EI+): 527 (M+1)

**Example 2c:** 5-(S)-[2-(S)-(1-Allyl-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl)-propionylamino]-4-(S)-hydroxy-2-(R)-methyl-6-phenyl-hexanoic acid butylamide

MS(EI+): 527 (M+1)

**Example 3:** 4-(S)-Hydroxy-5-(S)-{2-(S)-[1-(4-hydroxy-butyl)-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-7-yl]-propionylamino}-2-(R)-methyl-6-phenyl-hexanoic acid butylamide

30 mg (0.06 mmol) 4-(7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-1-yl)-butyric acid methyl ester are stirred overnight at room temperature in 1 mL butylamine. The reaction mixture is evaporated to dryness, taken up in toluene and evaporated again, and the residue dissolved in THF, cooled to 5°C and treated with 3 mg (2 eq.) lithium borohydride. After stirring for two hours at room temperature, the reaction mixture is cooled below 10°C, quenched with 2 mL of saturated aqueous ammonium chloride and 2 mL of saturated aqueous sodium bicarbonate and stirred an additional 10 minutes before extraction with EtOAc (twice). The combined organic phases are dried over sodium sulfate, evaporated, and the residue column chromatographed (silica gel, DCM/EtOH/ammonia 90:10:0.05) to yield the desired product as a light-colored resin.

MS(EI+): 559 (M+1)

The starting materials can be prepared as described hereafter:

a) 4-(7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenyl-ethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-1-yl)-butyric acid methyl ester

30 mg (0.06 mmol) 4-(7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenylethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-1-yl)-but-2-enoic acid methyl ester are stirred in THF under hydrogen for two hours in the presence of a catalytic amount of 10% Pd/C, then filtered through celite and evaporated to yield 30 mg desired product, which is used without further purification.

MS(EI+): 514 (M+1)

**b) 4-(7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenylethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]non-1-yl)-but-2-enoic acid methyl ester**

130 mg (0.25 mmol) 7-{1-(S)-[1-(S)-(4-(R)-Methyl-5-oxo-tetrahydro-furan-2-(S)-yl)-2-phenylethylcarbamoyl]-ethyl}-6-oxo-1,7-diaza-spiro-(R)-[4.4]nonane-1-carboxylic acid tert-butyl ester are dissolved in 2 mL of 4N HCl in dioxane. The reaction mixture is evaporated after 90 minutes, taken up in toluene and evaporated to dryness. The residue is taken up in dichloromethane and stirred at room temperature for 18 hours in the presence of 92 mg (1 eq.) tetrabutylammonium iodide, 0.03 mL trans-4-bromobut-2-enoic acid methyl ester and 0.09 mL (2 eq.) diisopropylethylamine. The reaction mixture is extracted with dichloromethane and aqueous bicarbonate (twice), the combined organic phases evaporated and the residue column chromatographed (silica gel, EtOAc) to yield the desired product as a slightly brownish resin.

MS(EI+): 512 (M+1)

The following compound is obtained by a similar procedure:

**Example 3a: 4-(S)-Hydroxy-5-(S)-{2-(S)-[1-(4-hydroxy-butyl)-6-oxo-1,7-diaza-spiro-(S)-[4.4]non-7-yl]-propionylamino}-2-(R)-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 559 (M+1)

**Example 4: 4-Hydroxy-5-{2-[1-(4-hydroxy-but-2-enyl)-6-oxo-1,7-diaza-spiro[4.4]non-7-yl]-propionylamino}-2-methyl-6-phenyl-hexanoic acid butylamide**

MS(EI+): 557 (M+1)

is obtained in a similar manner to example 1, but using 2-[1-(4-hydroxy-but-2-enyl)-6-oxo-1,7-diaza-spiro[4.4]non-7-yl]-propionic acid methyl ester in step 1a .

The starting material can be prepared as described hereafter:

**2-[1-(4-Hydroxy-but-2-enyl)-6-oxo-1,7-diaza-spiro[4.4]non-7-yl]-propionic acid methyl ester**

82 mg (0.25 mmol) (S,S)-7-(1-methoxycarbonyl-ethyl)-6-oxo-1,7-diaza-spiro[4.4]nonane-1-carboxylic acid tert-butyl ester are stirred at room temperature for three hours in 1 mL of a 4N HCl dioxane solution, evaporated, then taken up in toluene and evaporated again (twice). The residue is taken up in 2 mL dichloromethane and stirred at room temperature for 65 hours in the presence of 74 mg (0.2 mmol) tetrabutylammonium iodide, 0.035 mL (0.2 mmol) diisopropylethylamine and 30 mg (0.2 mmol) 4-bromo-but-2-en-1-ol. The reaction mixture is extracted with EtOAc and saturated aqueous sodium bicarbonate, the combined organic phases washed with brine, evaporated to dryness and the residue column chromatographed (silica gel, EtOAc/EtOH 9:1) to yield the desired product as a thick liquid.

MS(EI+): 297 (M+1)

**Example 5: (S)-N-[(1S,2R)-1-Benzyl-2-hydroxy-3-(3-isopropyl-benzylamino)-propyl]-2-((S)-6-oxo-1-propyl-1,7-diaza-spiro[4.4]non-7-yl)-propionamide**

A solution of 100 mg (2R,3S)-3-amino-1-(3-isopropyl-benzylamino)-4-phenyl-butan-2-ol dihydrochloride, 79 mg (S)-2-((S)-6-oxo-1-propyl-1,7-diaza-spiro[4.4]non-7-yl)-propionic acid, 102 mg TBTU and 0.171 mL N-methyl morpholine in 5 mL CH<sub>2</sub>Cl<sub>2</sub> is stirred for 5h at ambient temperature. The solution is diluted with DCM and subsequently washed with bicarbonate, brine, 0.1N HCl and bicarbonate. After drying with MgSO<sub>4</sub> all volatiles are evaporated in vacuo and the product is purified by column chromatography (silica gel, DCM/MeOH 95:5) to give 53 mg (37%) of the desired product.

MS-ESI+: 549 [M+]

Rf: 0.28 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 9/1)

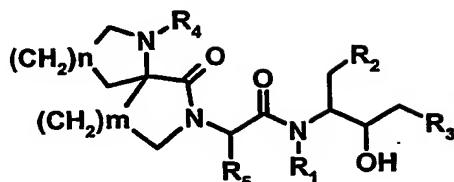
The starting material can be prepared as described hereafter:

**a) (2R,3S)-3-amino-1-(3-isopropyl-benzylamino)-4-phenyl-butan-2-ol dihydrochloride**

A solution of 700 mg (2.7 mmol) *tert*-butyl(S-(R,R)(-)-(1-oxiranyl-2-phenylethyl)-carbamate and 470 mg (3.3 mmol) 3-iso-propylbenzylamine in 10 ml EtOH is heated for 15 h at 50°C. Evaporation of the solvent and purification by column chromatography (silica gel, DCM/MeOH 9:1) afforded 820 mg of [(1S,2R)-1-benzyl-2-hydroxy-3-(3-iso-propyl-benzylamino)-propyl]-carbamic acid *tert*-butyl ester as a colourless solid. This material is dissolved in 10 ml 4N HCl in dioxane, stirred for 2 h at ambient temperature and all volatiles removed in vacuo to give 643 mg desired compound.

Claims:

1. A compound of formula I



wherein

$R_1$  is hydrogen or  $(C_{1-4})$ alkyl,

$R_2$  is optionally substituted  $(C_{1-8})$ alkyl,  $(C_{3-7})$ cycloalkyl,  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl, aryl or heteroaryl,

$R_3$  is  $CH(R_e)CONR_aR_b$  or  $(CH_2)_kNR_cR_d$ , wherein

$k$  is 0, 1 or 2,

$R_a$ ,  $R_b$ ,  $R_c$  and  $R_d$ , independently, are hydrogen or optionally substituted  $(C_{1-8})$ alkyl,  $(C_{3-7})$ cycloalkyl,  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl, aryl, aryl $(C_{1-4})$ alkyl, heteroaryl, heteroaryl $(C_{1-4})$ alkyl, 4-chromanyl, 1,2,3,4-tetrahydro-quinolin-4-yl, 1,2,3,4-tetrahydro-naphthalenyl, thiochroman-4-yl-1,1-dioxide, 4-isochromanyl, 1,2,3,4-tetrahydro-isoquinolin-4-yl, thioisochroman-4-yl-1,1-dioxide, 1,1-dioxo-1,2,3,4-tetrahydro-1 $\lambda^6$ -benzo[e][1,2]thiazin-4-yl, 1,1-dioxo-3,4-dihydro-1H-1 $\lambda^6$ -benzo[c][1,2]oxathiin-4-yl, 2,2-dioxo-1,2,3,4-tetrahydro-2 $\lambda^6$ -benzo[c][1,2]thiazin-4-yl or 2,2-dioxo-3,4-dihydro-2H-2 $\lambda^6$ -benzo[e][1,2]oxathiin-4-yl, or

$R_a$ , and  $R_b$ , or  $R_c$  and  $R_d$ , together with the nitrogen to which they are attached, form an optionally substituted pyrrolidiny, piperidino, morpholino or piperaziny group, and

$R_e$  is  $(C_{1-8})$ alkyl,  $(C_{1-4})$ alkoxy $(C_{1-4})$ alkyl,  $(C_{3-7})$ cycloalkyl or  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl,

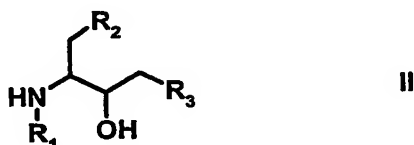
$R_4$  is hydrogen or optionally substituted  $(C_{1-8})$ alkyl,  $(C_{1-4})$ alkoxy $(C_{1-4})$ alkyl,  $(C_{3-7})$ cycloalkyl,  $(C_{3-7})$ cycloalkyl $(C_{1-4})$ alkyl,  $(C_{1-8})$ alkenyl or  $(C_{3-7})$ cycloalkoxy $(C_{1-4})$ alkyl,

$R_5$  is hydrogen or optionally substituted  $(C_{1-4})$ alkyl, and

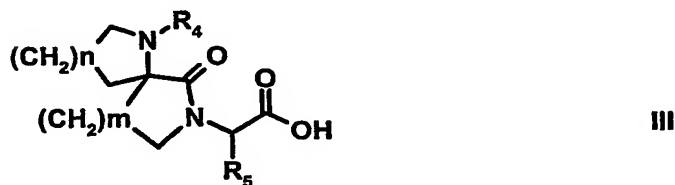
$m$  and  $n$ , independently, are 1 or 2

in free-base or acid-addition-salt form.

2. A process for the preparation of a compound of formula I as defined in claim 1, or a salt thereof, which includes the steps of acylating a compound of formula II



wherein  $R_1$ ,  $R_2$  and  $R_3$  are as defined in claim 1, with an acid of formula III



wherein  $R_4$ ,  $R_5$ ,  $m$  and  $n$  are as defined in claim 1, or an activated form thereof, and recovering the so obtained compound of formula I in free base or acid addition salt form.

3. A compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, for use as a pharmaceutical.
4. A compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, for use in the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation.
5. A pharmaceutical composition comprising a compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, in association with a pharmaceutical carrier or diluent.
6. The use of a compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, as a pharmaceutical, for the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation.

7. The use of a compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, for the manufacture of a medicament for the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation.
8. A method for the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation in a subject in need of such treatment, which comprises administering to such subject a therapeutically effective amount of a compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form.
9. A combination comprising a therapeutically effective amount of a compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form and a second drug substance, for simultaneous or sequential administration.

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